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# Effects of Broiler Feed Medications on *Salmonella*

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SUMMARY. This pilot analysis was conducted with data from 52 conventional grow-out broiler flocks in a prospective field observational study in the southeastern United States during 2003–2006. Each flock was sampled for *Salmonella* 1 wk before the end of grow-out, upon arrival at the processing plant, and during processing (prior to and immediately after carcass chilling). The broiler litter was sampled on the day of bird harvest. The grow-out feeding programs, including the medications delivered in feed, were surveyed with questionnaires completed by the broiler managers and feedmill managers. Each detail of the feeding program was tested for statistical association with the frequency of *Salmonella* in the flock at each sampling point, after accounting for variation in *Salmonella* frequency between the farms, broiler complexes, and companies. Significant associations were found between *Salmonella* frequency in the broiler flock pre- and postharvest and the inclusion of feeds containing individual coccidiostats and other antimicrobial growth promoters, days on feed, and total consumption of feeds containing these products, as well as with practices such as a mash feed and a nonmedicated withdrawal feed. The analysis provided testable hypotheses for how broiler feed medications impact the frequency of *Salmonella* in the flocks.

RESUMEN. Efectos de los medicamentos de pollo de engorde sobre *Salmonella*.

Este análisis piloto se llevó a cabo con datos de 52 parvadas convencionales de pollo de engorde en un estudio de campo observacional prospectivo en el sureste de los Estados Unidos durante los años 2003 al 2006. Se recolectaron muestras de cada parvada para *Salmonella* una semana antes del final del periodo engorde tanto a su llegada a la planta de procesamiento y durante el proceso (antes e inmediatamente después del enfriamiento de la canal). La cama de pollos de engorda fue muestreada en el día que las aves se llevaron a la planta de procesamiento. Los programas de engorda de alimentación, incluidos los medicamentos adicionados en el alimento, fueron encuestados mediante cuestionarios completados por los directores y gerentes de fábrica de alimentos para pollo de engorde. Cada detalle del programa de alimentación se puso a prueba para la asociación estadística con la frecuencia de *Salmonella* en la parvada, en cada punto de muestreo después de contabilizada la variación en la frecuencia de *Salmonella* entre las granjas de pollos de engorde, los complejos y las empresas. Se encontraron asociaciones significativas entre la frecuencia de *Salmonella* antes y después del envío de las aves a la planta de procesamiento y con la inclusión de alimentos que contenían coccidiostatos individuales y otros antibióticos promotores del crecimiento, número de días en que se administró el alimento y el consumo total de alimento que contenían estos productos, así como con las prácticas tales como la administración de alimento en harina y alimento de retiro no medicado. El análisis proporciona hipótesis comprobables del mecanismo por el cual los medicamentos en el alimento afectan la frecuencia de *Salmonella* en las parvadas.

Key words: *Salmonella*, broiler, feed, coccidiostat, growth-promoter, antimicrobial

Abbreviations: BPW = buffered peptone water; RV = Rappaport-Vassiliadis; TET = tetrathionate; WCR = whole feathered carcass rinse

Grow-out feeding programs in the U.S. broiler industry are based on common approaches such as feed pelletization and usage of purposefully designed rations that include starter, grow-out, and withdrawal feeds. The feed is also used as a vehicle for delivering to the birds disease-preventive medications such as coccidiostats and other growth promoting antimicrobials. The actual feeding program varies flock-to-flock due to managerial decisions at the level of the individual broiler integrator and complex. These decisions are based on current knowledge of broiler nutrition and needs of disease prevention in the flocks. In-feed medications may impact *Salmonella* distribution in the grow-out flocks; in particular, in-feed antibiotics have been reported to impact *Salmonella* status of flocks reared in European (5,7,9) and African (3) production systems. In this study, we tested the significance of associations of in-feed medications and other details of the feeding program with *Salmonella* frequency in 52 conventional grow-out broiler flocks reared in the southeastern United States during 2003–2006.

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## MATERIALS AND METHODS

**Sampling.** Seventy conventional grow-out broiler flocks (2 flocks per farm) were sampled 1 wk before the end of rearing and upon arrival at the processing plant by a convenience sample of 30 broilers per flock. The collection of samples from the birds received approval from the Mississippi State University Institutional Animal Use and Care Committee (IACUC protocol no. 02-040, 2002). The sample collection and processing have been previously described in detail (13). In short, each broiler was humanely euthanatized by cervical dislocation, and the carcass was shaken for 1 minute in a bio-hazard bag with 250 ml of sterile buffered peptone water (BPW) to obtain the whole carcass rinse, which was transferred into a sterile plastic bottle and incubated at 42 C overnight. The carcass was then opened aseptically; the crop was removed and placed in a sterile Whirl-Pak® Filter Bag (Nasco, Fort Atkinson, WI), and one cecum was removed and placed in a sterile Whirl-Pak bag. Initial processing of these samples was performed immediately. The cecum was weighed, and nine times the weight of tetrathionate (TET) broth (Remel, Lenexa, KS) was added, stomached for 60 s, and incubated at 42 C overnight. To the crop sample, nine times the weight of BPW was added, stomached for 60 s, and incubated at 42 C overnight.

During processing of the flock, 30 carcasses were taken immediately before and 30 immediately after the immersion chill tank, and a rinse of

each carcass was obtained. The first carcass was taken at the beginning of the flock passing through that particular sampling point, and the rest at even time intervals across the flock processing time at the point. New gloves were used to take each carcass from the line, which was placed in a sterile plastic bag with 100 ml of Butterfield's solution, and shaken for 1 min. The rinse was aseptically transferred into a sterile plastic bottle; concentrated BPW was added to bring the final concentration to single-strength BPW, and incubated at 42 C overnight.

Four pooled broiler litter samples and four drag swabs of the litter were collected from the grow-out house within 4 hr after sampling the birds at the processing plant. Each swab was made with cotton gauze (Abco Dealers, Nashville, TN) that was tied to a cotton–polyester string (Lehigh Group, Macungie, PA). These were steam-sterilized and aseptically transferred to a sterile Whirl-Pak bag containing 20 ml of sterile double-strength skim milk. The four samples of each type were collected to sample the entire breadth and width of the house floor. Samples were transported to the laboratory within 8 hr of collection, where they were enriched employing a previously validated methodology (10). In particular, 25 g of a litter sample was placed into a Whirl-Pak bag, 225 ml of BPW was added, mixed for 1 min, and incubated at 42 C overnight. To each drag swab, 100 ml of BPW was added, mixed, and incubated at 42 C overnight.

The sample size of 30 broilers/carcasses per flock allowed for at least one expected *Salmonella*-positive sample at that sampling point in a flock where within-flock *Salmonella* prevalence was greater than the U.S. national average at the time of the study design, 9.5% (2,11). The number of pooled litter samples and drag swabs was chosen based on the experience of the research team.

Each sample was analyzed for presence of *Salmonella* using standard microbiological techniques (10,13). A pre-enriched (environmental) or a raw (biological) sample, 1 ml, was added to TET broth (Remel) for 48-hr incubation at 42 C. After incubation, 0.1 ml sample of the TET was transferred to Rappaport-Vassiliadis (RV) broth (Difco Laboratories, Detroit, MI) for overnight incubation at 42 C. Then one loopful of the RV was plated onto a Xylose-Lysine-Tergitol 4 (XLT4) agar plate (Remel) for overnight incubation at 37 C. A single colony was picked from a positive XLT4 plate, and *Salmonella* isolation was confirmed biochemically, and in a slide agglutination assay with *Salmonella* O Antiserum Poly A-I & Vi (Difco) as described by the manufacturer.

**Collection of feeding program data.** For 52 of the 70 sampled flocks, the feedmill managers completed a Feedmill Manager questionnaire, and the broiler managers completed a Broiler Manager questionnaire that included a table to systematically describe the grow-out feeding program (12). Therefore the survey response rate was 74%. Both questionnaires were designed for the study and were pilot-tested with industry personnel in the study region (12). The questionnaires were administered in paper form, and the responses were entered and stored in a Microsoft Office Access 2003® database (Microsoft, Redmonds, WA). The survey was reviewed and approved by the Mississippi State University Institutional Review Board (IRB protocol no. 04-005, 2004).

**Demographics of studied flocks.** The broilers were, on average, 8 wk old at harvest, and each flock consisted of between 15,200 and 27,200 birds. The 52 analyzed flocks were reared on 26 grow-out farms managed by eight broiler complexes owned by two companies.

**Statistical analysis.** Seven feedmills produced the feeds used for the 52 flocks. Identity of the feedmill and 46 details of the feeding program (including details of in-feed administration of individual coccidiostats and growth promoters) were each tested for significance of statistical association with *Salmonella* frequency in the flock as measured by each of the sample types at each of the sampling points. Logistic regression was used, with the model outcome being the proportion of *Salmonella*-positive samples of the type collected at the sampling point. The model was fitted as a generalized linear mixed model; the association between the outcome and a feeding program's detail was tested after accounting for variation in *Salmonella* frequency due to the random effects of the farms, complexes, and companies. The association was considered as statistically significant if $P \leq 0.10$ for the detail; this level of significance was chosen because of the limited number of flocks analyzed for each program's detail. For the same reason, a multipredictor model was not fitted. The assumption of linear relationships between each continuous predictor and the relevant outcome was assessed using two methods. First, a logistic regression model with dummy variables for the predictor's quartiles was fitted; the linearity in the plot of the predictor's regression coefficient *vs.* the midpoints of its quartiles was evaluated visually (8). Second, with all the data for the predictor/outcome, LOWESS plots of the logit *vs.* the predictor values were created, and evaluated visually.

The statistical analysis was implemented in SAS 9.1® software for Windows (SAS Institute, Cary, NC), except for the two methods used to evaluate the linearity of relationships between continuous predictors and the relevant outcomes. These were implemented in Stata 12.1 software (StataCorp, College Station, TX); the LINCHECK module (6) was used for the quartile analysis.

## RESULTS

Statistically significant associations were observed between *Salmonella* frequency in the flock and several details of the grow-out feeding program. A medication delivered in feed was classified as a coccidiostat or as growth promoter in accordance with the primary purpose of its usage as a broiler feed additive. The usage of the coccidiostat narasin in feed and its duration and pounds per bird of feeds containing it, especially in combination with the coccidiostat nicarbazin, were consistently associated with lower *Salmonella* frequencies in the flock pre- and postharvest (Table 1). The results were similar for in-feed delivery of another drug used for coccidiosis control and as a growth promoter, roxarsone. Similarly, a higher number of days with feed containing the growth promoter bacitracin methylene disalicylate was associated with a lower frequency of broilers with *Salmonella* in the ceca during grow-out (Table 1). Conversely, administration of the coccidiostat salinomycin sodium was associated with a higher frequency of *Salmonella*-contaminated broiler carcasses postharvest (Table 1).

Of the overall design of the feeding program, usage of a mash feed was associated with higher *Salmonella* frequencies on the broiler carcasses postharvest (Table 2). A higher consumption, in pounds per bird, of any starter feed (mash or crumbles) was associated with higher *Salmonella* frequencies in the flock at arrival for processing and postharvest (Table 2). A higher total number of different feed compositions delivered during grow-out was a risk for the pre- and postharvest *Salmonella* outcomes (Table 2). Administration of a nonmedicated withdrawal feed at the end of grow-out, and a higher consumption of this feed were associated with reduced *Salmonella* frequency in the broiler ceca at arrival for processing (Table 2).

Of the other data collected, the average reported maximum temperature of feed pelletization was 87.5 C (range 85.0–92.2 C). The average reported time from the production of the pelleted feed to its delivery to the farm was 16.5 hr (range 3–48 hr). No probiotic was added to the feeds for any of the flocks reporting ($n = 48$ responses). Administration of supplements (those trademarked Alimet and Betaine were reported) was not associated with *Salmonella* frequency in the flock.

## DISCUSSION

Our findings indicated that in-feed administration of antimicrobials may affect *Salmonella* frequency in the broiler flocks during grow-out; this concurs with previous reports (3,5,7,9). However, the comparison may be complicated by the fact that in our study the broilers averaged

Table 1. In-feed medications administered to grow-out broilers that were associated ($P \leq 0.10$) with *Salmonella* frequency in the flock. A medication was classified as a coccidiostat or growth promoter in accordance with the primary purpose of its usage as broiler feed additive.

| Risk factor | Increment modeled | Flock count or mean (range) | Sample type with which *Salmonella* frequency was measured | OR (Wald-type 95% CI) | *P* |
|---|---|---|---|---|---|
| Coccidiostats nicarbazin and narasin in feed (together) | Yes | 18 | Ceca during grow-out | 0.32 (0.08, 1.24) | 0.095 |
| | No (reference) | 34 | | | |
| | Yes | 18 | Crop during grow-out | 0.32 (0.10, 1.24) | 0.095 |
| | No (reference) | 34 | | | |
| | Yes | 18 | Drag swab of grow-out litter | 0.31 (0.07, 1.24) | 0.094 |
| | No (reference) | 32 | | | |
| | Yes | 18 | WCR[A] at arrival for processing | 0.28 (0.11, 0.73) | 0.011 |
| | No (reference) | 30 | | | |
| | Yes | 18 | Prechill carcass rinse | 0.21 (0.042, 1.04) | 0.055 |
| | No (reference) | 30 | | | |
| Days on feed with narasin (alone or with nicarbazin) | Days delivered | 21.9 (0–63) | WCR during grow-out | 0.97 (0.94, 1.00) | 0.044 |
| | | | WCR at arrival for processing | 0.98 (0.96, 1.00) | 0.066 |
| Consumption of feed with narasin (alone or with nicarbazin) | Pounds per bird | 4.8 (0–14.4) | WCR during grow-out | 0.86 (0.75, 0.99) | 0.033 |
| | | | WCR at arrival for processing | 0.90 (0.82, 0.99) | 0.028 |
| Days on feed with narasin (alone) | Days delivered | 13.5 (0–49) | WCR during grow-out | 0.95 (0.91, 0.99) | 0.013 |
| | | | WCR at arrival for processing | 0.97 (0.94, 1.00) | 0.024 |
| | | | Postchill carcass rinse | 0.95 (0.91, 1.00) | 0.032 |
| Consumption of feed with narasin (alone) | Pounds per bird | 4.0 (1–13.4) | WCR during grow-out | 0.82 (0.71, 0.96) | 0.016 |
| | | | WCR at arrival for processing | 0.87 (0.79, 0.97) | 0.013 |
| | | | Postchill carcass rinse | 0.85 (0.73, 0.99) | 0.037 |
| Coccidiostat salinomycin sodium in feed | Yes | 16 | Prechill carcass rinse | 6.52 (1.35, 31.40) | 0.021 |
| | No (reference) | 32 | | | |
| Days on feed with salinomycin sodium | Days delivered | 10 (0–50) | Prechill carcass rinse | 1.04 (0.99, 1.08) | 0.093 |
| Coccidiostat and growth promoter roxarsone in feed | Yes | 40 | Ceca at arrival for processing | 0.26 (0.06, 1.17) | 0.078 |
| | No (reference) | 8 | | | |
| Consumption of feed with roxarsone | Pounds per bird | 4.8 (0–11.0) | WCR during grow-out | 0.80 (0.63, 1.02) | 0.067 |
| | | | WCR at arrival for processing | 0.84 (0.73, 0.97) | 0.020 |
| | | | Postchill carcass rinse | 0.81 (0.66, 1.00) | 0.053 |
| Days on feed with growth promoter BMD[B] | Days delivered | | Ceca during grow-out | | 0.038 |
| | 46–60 | 14 | | 0.14 (0.03–0.61) | |
| | 31–45 | 6 | | 0.63 (0.10–3.93) | |
| | 16–30 | 14 | | 0.24 (0.05–1.05) | |
| | 0–15 (reference) | 36 | | | |

[A]WCR = whole feathered carcass rinse.
[B]BMD = bacitracin methylene disalicylate.

7 wk of age when sampled during grow-out, while sampled broilers were younger in the European and African production systems previously surveyed. Unique to our study, these associations were found to hold until postharvest, showing that in-feed medications impact the frequency of *Salmonella*-contaminated broiler carcasses after the flock is processed (Table 1). The tendency appears to be that in-feed antimicrobial growth promoters lead to a lower *Salmonella* frequency in the flock; however, this may differ between individual drugs and specific outcomes measured. For example, there was a positive association between in-feed administration of salinomycin sodium and frequency of *Salmonella*-contaminated carcasses in the flock postharvest (Table 1). The limited number of flocks analyzed prevents us from making more definitive conclusions for individual drugs.

The mechanism underlying the associations between in-feed antimicrobial growth promoters and *Salmonella* may be a direct action of the drugs on intestinal *Salmonella*. With this mechanism, it might have been that the reduction in *Salmonella* frequency was achieved by lowering the number of *Salmonella* sensitive to the drugs. The sensitivity of isolated *Salmonella* to the antimicrobials administered was not measured in this study. However, the mechanism leading to lower *Salmonella* frequencies may also be indirect: the drugs may alter the structure or diversity of the intestinal microbial community, therefore altering the size of niche available for *Salmonella*. Overall, the mechanism is not necessarily related to the broiler-growth–promoting effect of the drugs, as there was no significant association between the final live broiler weight or grow-out feed conversion and *Salmonella* frequencies. These two observations of no association, however, should be considered with caution in that they may suffer from ecological fallacy: the live weight and feed conversion were calculated across all the flocks reared on the farm in the all-in/all-out grow-out cycle with the two sampled flocks, while *Salmonella* frequencies were measured on the flock level.

We also found significant associations between in-feed delivery of coccidiostats and *Salmonella* frequencies in the broilers during rearing, in the grow-out litter, and on the carcasses (Table 1). Our results suggest that the direction of the effect, more or less *Salmonella* present, may be drug-specific (Table 1). It is possible that the effect depends on the degree of sensitivity of *Eimeria* species or strains circulating in the flock to the coccidiostat delivered. Our previous investigation showed that the *Salmonella* burden in a grow-out flock may depend on the design and efficacy of the *Eimeria* control program (14).

In terms of the overall design of the grow-out feeding program, using a mash feed was a risk factor associated with higher *Salmonella*

Table 2. General design details of the grow-out feeding program that were associated ($P \leq 0.10$) with *Salmonella* frequency in the flock.

| Risk factor | Increment modeled | Flock count or mean (range) | Sample type with which *Salmonella* frequency was measured | OR (Wald-type 95% CI) | *P* |
|---|---|---|---|---|---|
| Mash feed | Yes | 8 | Prechill carcass rinse | 6.71 (0.66, 67.60) | 0.101 |
| | No (reference) | 32 | | | |
| | Yes | 8 | Postchill carcass rinse | 7.66 (2.23, 26.30) | 0.003 |
| | No (reference) | 30 | | | |
| Consumption of starter feed | Pounds per bird | 1.3 (1–2) | Crop at arrival for processing | 4.69 (0.72, 30.60) | 0.100 |
| | | | Ceca at arrival for processing | 10.90 (1.47, 80.90) | 0.022 |
| | | | Postchill carcass rinse | 9.37 (1.37, 640) | 0.023 |
| Number of feed compositions during grow-out | Number | 4 (3–5) | WCR[A] during grow-out | 14.50 (1.46, 145) | 0.024 |
| | | | Drag swab of grow-out litter | 9.89 (0.80, 119.00) | 0.070 |
| | | | Prechill carcass rinse | 12.80 (1.02, 160) | 0.048 |
| Nonmedicated withdrawal feed at the end of rearing | Yes | 34 | Ceca at arrival for processing | 0.20 (0.05, 0.77) | 0.022 |
| | No (reference) | 10 | | | |
| Consumption of nonmedicated withdrawal feed | Pounds per bird | 2.3 (0–5.2) | Ceca at arrival for processing | 0.67 (0.47, 0.95) | 0.026 |

[A]WCR = whole feathered carcass rinse.

frequencies in the flock at postharvest (Table 2). A mash feed does not undergo the high temperatures used in manufacturing pelleted feed and so may be more likely to harbor *Salmonella* and serve as a vehicle of broiler exposure. Broiler exposure to *Salmonella* in the first days of life leads to a high prevalence later (1,4).

A higher total number of feeds delivered to the flock during grow-out was a risk factor for both the pre- and postharvest *Salmonella* outcomes (Table 2). This suggests that adaptation of birds to a change in feed composition may impact on *Salmonella* frequency. However, the observation could have resulted from confounding, as a higher number of feeds could be in the flocks fed a mash feed. An alternative explanation may be that with a higher variety of feeds, there was more feed deliveries during the grow-out. The more extensive traffic may have increased the likelihood of *Salmonella* introduction if the vehicles or driver's footwear had become contaminated.

Usage and pounds per bird of a nonmedicated withdrawal feed at the end of rearing were associated with a significant reduction in proportion of broilers having *Salmonella* in the cecum at the time of arrival at the processing plant in this study (Table 2). This may have been due to a change in pH of the cecal contents.

The data analyzed were collected in the southeastern United States during 2003–2006. The overall design of the grow-out feeding program in the region has not changed from that time till now. However, several details of the program have. First, the sales of roxarsone for feed medication have been suspended by the producer. Another new practice is that those integrators choosing to control broiler coccidiosis through vaccination sometimes also administer in-feed ionophores to the flocks. Finally, because of the increasing price of finished broiler feed, distillers dried grains and potentially lower quality ingredients have been used as feedstuffs.

This analysis provided testable hypotheses for how broiler feed medications impact the frequency of *Salmonella* in the flock pre- and postharvest. These hypotheses can be followed up in detailed field or experimental investigations.

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